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1. Conner et al. (1983) PNAS 80: 278-282.
2. Rollini et al. PNAS (1985 Nov) 82(12): 7197-7201.
3. Gorski et al. IMMUNOGENTICS (1987) 25(6):379-402.
4.. de Preval et al. IMMUNOGEENTICS (1987) 26(4-5): 249-257.
5. Irle et al. J. EXPERIMENTAL MEDICINE (1988 Mar 1) 167(3): 853-872.
6. Andersson et al. IMMUNOGENETICS (1988) 28 (1): 1-5.

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Immuno-genetics

Structural Comparison of the Genes of Two HLA-DR Supertypic Groups: The Loci Encoding DRw52 and DRw53 Are Not Truly Allelic

Jack Gorski*, Pierre Rollini, and Bernard Mach

Department of Microbiology, University of Geneva Medical School, 1211 Geneva, Switzerland

Abstract. The organization and sequence of the *HLA-DR*$_\beta$ chain genes are compared in the two supertypic groups, DRw52 and DRw53, which together account for more than 80% of *HLA-DR* alleles. From the structural data, we conclude that these two groups represent distinct lineages which have followed different patterns of evolution. The fine structure of the β chain locus encoding the DRw53 specificity corresponds most closely to the *DR*$_\beta$*II* pseudogene in the *DRw52* haplotypes. Concomitantly, the *DR*$_\beta$*I* locus in *DRw53* haplotypes is more closely related to both of the two expressed *DR*$_\beta$ loci of the *DRw52* haplotypes (*DR*$_\beta$*I* and *DR*$_\beta$ *III*). These two loci are the result of a recent duplication. This leads to the proposal that both expressed *DR*$_\beta$ chain genes in the *DRw52* haplotypes (*DR*$_\beta$*I* and *DR*$_\beta$*III*) are derived from a single precursor locus, while the two loci expressed in the *DRw53* haplotypes are derived from distinct ancestral loci. The genes encoding DRw52 and DRw53 are therefore not true alleles of the same original locus. A scheme is proposed that accounts for the evolution of DR specificities within the DRw52 and DRw53 groups of haplotypes. It is evident that the different *HLA-DR* alleles are not structurally equidistant and that one must take into consideration different degrees of heterozygosity or mismatch among the *DR* alleles.

Introduction

A striking feature of the major histocompatibility complex (MHC) is its remarkable allelic polymorphism. This polymorphism is responsible for the phenomenon of restriction of antigen presentation to T lymphocytes of the same haplotype. The class II products of the MHC are involved in presentation of antigen to a subset of T lymphocytes. Different class II allelic products can present certain antigens with either low or high efficiency, thus determining the extent of the immune response (reviewed by Benacerraf 1981, Nagy et al. 1981, Schwartz 1985). The biological significance of MHC class II polymorphism is the ability it confers to the population to cope with a variety of pathogens and thus to survive as a species.

The major class II product of the human MHC (*HLA*) is HLA-DR. This transmembrane protein is composed of two chains, the α and β chains. The α chain is encoded by a single locus (*A*) and shows no polymorphism. The β chains, on the contrary, are highly polymorphic and are encoded by several (*B*) loci (reviewed by Kaufman et al. 1984, Rollini et al. 1985). The correlation between the products of each of these individual β chain loci and the observed phenotypic traits such as serologic reactivity, alloreactive T-cell stimulation and restricted antigen presentation represents an important challenge.

HLA-DR was first defined by serology. Sera directed against HLA-D/DR molecules often recognize the products of several different alleles and are thus designated as supertypic sera. They define groups of cross-reactive haplotypes called "supertypic groups." Two major supertypic groups have been identified. The members of the DRw52 supertypic group, haplotypes *DR3*, *DR5*, *DRw6*, and *DRw8*, share the DRw52 specificity. The members of the DRw53 group, *DR4*, *DR7*, and *DRw9*, share a specificity referred to as DRw53 (Bodmer and Bodmer 1984). Together these haplotypes account for more than 80% of known DR specificities. The epitopes recognized by supertypic sera could be encoded by a single *DR*$_\beta$ chain locus or by conserved regions shared by several β chain loci.

The identification of multiple *HLA-DR*$_\beta$ chain loci (Long et al. 1983) and the possibility of nonequal rates of divergence of these loci led us to propose that a supertypic epitope could be encoded by a less polymorphic locus shared among several haplotypes (Gorski et al. 1985). Analysis of one of the *HLA-DR*$_\beta$ chain genes expressed in DNA-transfected mouse fibroblasts has shown directly that it encodes the DRw52 specificity (Gorski et al. 1985). This gene has now been identified as the *DR*$_\beta$*III* locus of a

* *Present address:* The Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin

DRw52 haplotype (Rollini et al. 1985). In a *DRw53* haplotype, amino acid sequence analysis of the DRw53 reactive product and the nucleotide sequence of genes cloned from a *DR4* haplotype have permitted the identification of one of the DR_β chain loci as encoding DRw53 (Sorrentino et al. 1985, Spies et al. 1985). It is generally assumed that DRw52 and DRw53 are encoded by alleles of the same locus (Bach 1985).

In this study, we have analyzed the structural relationship of the β chain genes of the DRw52 and DRw53 supertypic groups at the level of fine restriction maps and of DNA sequence. We can conclude that the two expressed *HLA-DR*$_\beta$ chain loci in the *DRw52* haplotypes ($DR_\beta I$ and $DR_\beta III$) are homologous to the $DR_\beta I$ locus of the *DRw53* haplotypes, and that these three genes are presumably derived from a common ancestral locus, $DR_\beta I$. In contrast, the gene encoding the DRw53 specificity is distinct from these three genes and is most related to the $DR_\beta II$ pseudogene of the *DRw52* haplotypes. These two genes are presumably derived from another ancestral locus. The data suggest an evolutionary scheme for the *HLA-DR*$_\beta$ chain region, where the genes encoding DRw52 and DRw53 are not alleles of the same ancestral locus. They also have practical implications concerning the nature and the magnitude of the structural differences among different *HLA-DR* alleles.

Materials and Methods

Isolation and nomenclature of DR_β chain genes. The isolation and mapping of the DR_β chain genomic clones for the *DR3* and *DRw6* haplotypes have been described (Rollini et al. 1985). cDNA clones from a DR4,w6 cell line have also been published (Long et al. 1983). The DR4/DRw53 genomic clone was isolated from a phage library from the same DR4,w6 cell line. Four overlapping phage clones were isolated. The organization of the three $DR3_\beta$ chain genes has been described (Rollini et al. 1985). These loci are numbered in the direction of transcription using Roman numerals. The terms DRw52 supertypic group and DRw53 supertypic group are used to define the evolutionarily related groups of haplotypes sharing the DRw52 and DRw53 specificities, respectively. The terms *DRw52* locus and *DRw53* locus refer to the transcriptionally active locus which carries the DRw52 or DRw53 supertypic specificity and not the DR specificity. In DRw52, where the three DR_β loci have been linked, they are logically identified according to the gene order ($DR_\beta I$, $DR_\beta II$, $DR_\beta III$). In the case of *DR4*, however, all β chain loci have not yet been linked (Spies et al. 1985), and since four DR_β chain loci have been identified in certain *DRw53* haplotypes (Böhme et al. 1985), the locus encoding DRw53 might in fact correspond to a $DR_\beta IV$ locus (see *Discussion*).

Oligonucleotide blot hybridization. The oligonucleotides, 19mers, were kindly provided by Dr. E. Kawashima (Biogen S.A.) or synthesized using a Pharmacia Gene Assembler. The sequences which correspond to the different probes used are shown in Figure 1. The experimental protocol for the use of oligonucleotides under conditions of discrimination of single nucleotides is published (Angelini et al. 1986).

Mapping of genomic clones. The restriction enzyme maps presented here correspond to a fine level structural analysis of the genes. Mapping was performed by the classic single and double digestion techniques followed by Southern blot analysis to localize the exons by hybridization with cDNA probes. Position of the exons is based on the maps of subclones used for sequencing the respective exons.

Sequence analysis. The DR3, DRw6a, and DRw6b first-domain sequences used for the sequence comparison are published elsewhere (Gorski and Mach 1986). The first domain sequences of the *DRw53* gene from a *DR4* haplotype was determined from the genomic DR4 clone and from its corresponding cDNA (cDNA $DR_\beta IV$ in Long et al. 1983): The sequence for the $DR4_\beta I$ chain is from cDNA clone $DR_\beta II$ in Long et al. 1983 (B. Grubenmann et al., unpublished data). The same sequences have been recently published from other DR4 cell lines (Spies et al. 1985, Gregersen et al. 1986a).

Results

Identification of the DRw53 locus by oligonucleotide Southern blot hybridization. The *DRw53* locus has been identified by comparison of partial amino acid sequence of a protein precipitated with anti-DRw53 sera with the DNA sequence of a gene from a *DR4* haplotype (Sorrentino et al. 1985, Spies et al. 1985). We have determined the DNA sequence of the two expressed $DR4_\beta$ chain genes cloned from a DR4/w6 cell line and on the basis of these sequences, prepared oligonucleotide probes (4/1 and 4/2) which allow the unambiguous identification of one or the other locus (Fig. 1). The use of such 19 bp long oligonucleotides as probes to identify specific *DR* loci or specific *DR* alleles has been described in detail (Angelini et al. 1986). Probe 4/1 has been previously shown to hybridize only to DNA from DR4 individuals and thus identifies the corresponding sequence as that of the *DR4* haplotypic locus (Angelini et al. 1986). In contrast, probe 4-2 hybridizes to DNA from cells of the *DR4* and *DR7* haplotypes, both members of the DRw53 supertypic group. Another pair of locus-specific probes (4/3 and 4/4) corresponding to another region of the first domain (see Fig. 1) showed identical results (data not shown). Therefore, the β chain gene from which the sequences of probes 4/2 and 4/4 were

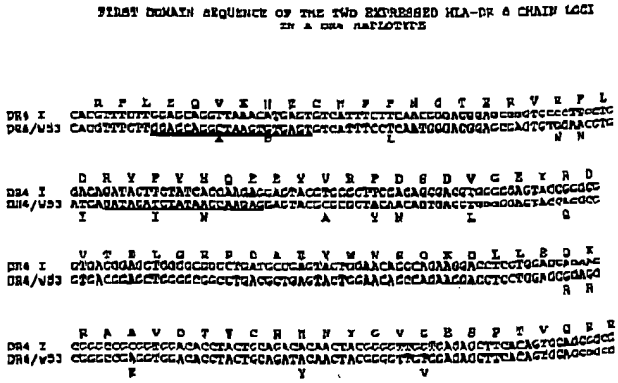

Fig. 1. First-domain sequence of $DR4_\beta I$ and DR4/DRw53. The region from which the oligonucleotide probes 4/1 and 4/2 are derived is *underlined*. The region corresponding to probes 4/3 and 4/4 is *underlined twice*

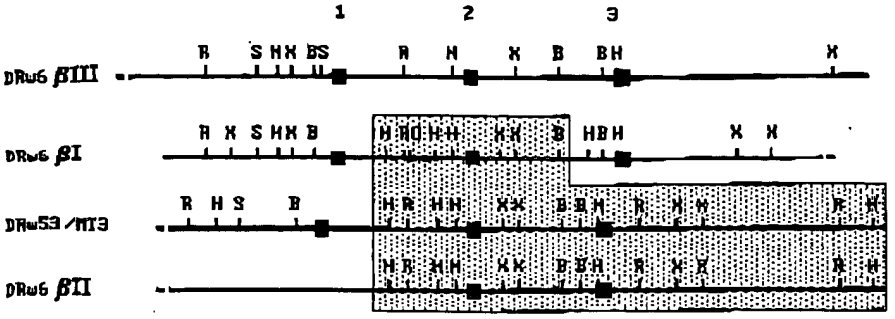

Fig. 2. Comparison of the maps of the three *DRw6* loci with the map of the *DR4/DRw53* locus. Sites for the following restriction enzymes are shown: B, Bam HI; H, Hind III; O, Xho I; R, Eco RI; S, Sac I; X, Xba. Regions of high homology are *boxed*. The *black boxes* refer to the exons encoding, respectively: 1, first domain; 2, second domain; 3, 3′ UT

derived corresponds to the locus encoding the DRw53 supertypic specificity.

The gene encoding the DRw53 specificity is more related to the $DR_\beta II$ pseudogene than to any other DR_β genes of the DRw52 haplotypes. In the *DRw52* haplotypes we have established a linkage map of three DR_β chain loci referred to as $DR_\beta I$, $DR_\beta II$, and $DR_\beta III$ (Rollini et al. 1985). The $DR_\beta I$ and $DR_\beta III$ loci are expressed whereas the $DR_\beta II$ locus lacks the first-domain exon and is thus considered a pseudogene (Rollini et al. 1985, 1987). Detailed restriction maps were generated for the three $DRw6_\beta$ chain loci.

A detailed map of the *DRw53* locus identified above was also prepared. It corresponds well to the map of the gene identified as *DRw53* on the basis of partial amino acid sequence (Sorrentino et al. 1985). This *DRw53* gene map was compared with maps of the three DR_β chain loci from the *DRw6* haplotype, $DR_\beta I$, $DR_\beta II$, and $DR_\beta III$ (Fig. 2). As can be seen from the comparison, the best homology between the *DRw53* locus and any of the *DRw6* loci is between DRw53 and $DRw6_\beta II$. The homology between the two restriction maps extends well downstream of the exons encoding the protein itself and covers a region known to contain repetitive sequences. Similarity of noncoding regions of DNA, which are not likely to be under selective pressure, is indicative of an ancestral relationship.

The $DR4_\beta I$ locus is more related to the two expressed DRw52 loci than it is to the locus encoding DRw53. The relationship between the genes of the DRw52 and DRw53 supertypic families was further analyzed by comparison of nucleotide sequences. It had been shown earlier that DR polymorphism is mostly associated with the first domain of the polypeptide chain (Kaufman and Strominger 1982). The sequence of the first-domain exons of the $DR4_\beta I$ and *DR4-DRw53* loci are shown in Figure 1. These were compared with the first-domain exon sequences of the $DR_\beta I$ and $DR_\beta III$ (*DRw52*) loci of the *DR3*, *DRw6a*, and *DRw6b* haplotypes, all members of the DRw52 supertypic group. Table 1 shows the number of amino acid differences, with the numbers in parentheses corresponding to nucleotide differences. It is clear that the $DR4_\beta I$ locus is equally related to both the $DR_\beta I$ and $DR_\beta III$ loci of haplotypes within the DRw52 supertypic group and that it is more related to these loci than it is to the *DR4-DRw53* locus.

The distribution of allelic differences at locus $DR_\beta I$ and at the supertypic locus $DR_\beta III$. Comparison of DNA sequences from the first domain of DR_β chain genes also permits an analysis of the distribution of allelic differences. This analysis can shed light on which regions of the DR_β chain may be involved in contact with antigen and/or the T-cell receptor. It also indicates where polymorphic sites may influence recognition by serologic reagents. When all DR gene sequences are compared in a nonallelic manner and irrespective of supertypic boundaries, a pattern is seen (Figure 3A) where several major regions of sequence variability can be identified (bp 15–25; bp 65–100; bp 155–165; and bp 195–220). However, comparisons made at one given locus and within a supertypic family show a very different distribution of polymorphic sites. The simplest case is the series of *DR4* alleles at the $DR_\beta I$

Table 1. Comparison of first domain sequences of DR_β chain genes

DR locus	$DR_\beta I$	$DRw6a_\beta I$	$DRw6b_\beta I$	$DR3_\beta III$ (*DRw52a*)	$DRw6b_\beta III$ (*DRw52b*)	$DR4_\beta I$
$DR4_\beta I$	13 (22)	12 (21)	13 (22)	13 (21)	13 (20)	0
DR4/DRw53	23 (31)	22 (34)	20 (28)	24 (34)	21 (31)	18 (24)

Results of comparisons of first domain sequences of three haplotypes within the DRw52 group with the $DR_\beta I$ and DRw53 locus sequences. Allelic comparisons within the DRw52 group are shown for $DR_\beta I$ (DRw6a and b) and for $DR_\beta III$ (DRw52a and b). Numbers represent amino acid differences; numbers in parentheses indicate base pairs

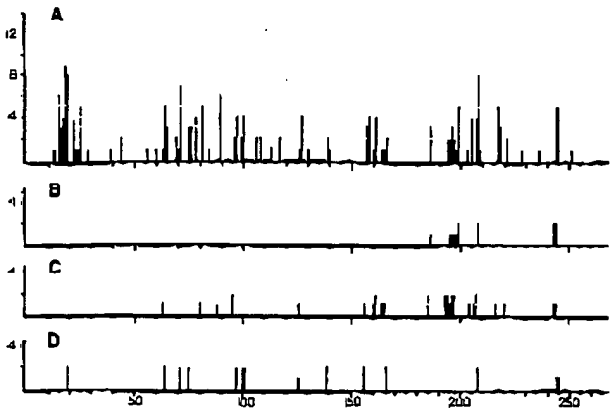

Fig. 3 A–D. Plot of distribution of polymorphic nucleotide sites in the first-domain encoding region. A Comparison of a number of *HLA-DR$_\beta$* chain genes irrespective of locus or supertypic group. The sequences used are from those presented here and those cited below as well as others previously published (Tonnelle et al. 1985, Gustafsson et al. 1984). B Comparison of alleles at the *DR$_\beta$I* locus of a number of DR4 cell lines (Cairns et al. 1985, Gregersen et al. 1986a). C Comparison of alleles at the *DR$_\beta$I* locus of a number of *DRw52* haplotypes (Gorski and Mach 1986, Ticher et al. 1986). D Comparison of alleles at the *DR$_\beta$III* locus of a number of *DRw52* haplotypes (Gorski and Mach 1986, Didier et al. 1986). The height of the line indicates the frequency of the second most common nucleotide. An *interrupted line* indicates that more than two nucleotides are found at that position and the length of each part of the line corresponds to the frequency of one of the nucleotides

locus (Fig. 3B). These alleles are all recognized as DR4 by serology and are only distinguished by alloreactive T-cell reagents defining Dw specificities. As has been pointed out previously (Cairns et al. 1985, Gregersen et al. 1986a), these differences are found predominantly in the 185–210 bp region. When the alleles at the *DR$_\beta$I* locus of different haplotypes of the DRw52 supertypic family are analyzed (Fig. 3C), the 185–210 bp region is also the most polymorphic segment, although some polymorphism is evident in the 155–165 bp region. In contrast and surprisingly, the alleles at the *DR$_\beta$III* locus of the DRw52 supertypic group show a very different distribution pattern from those observed at the *DR$_\beta$I* locus of either the DRw52 or DRw53 supertypic group (Fig. 3D), with almost no variation in the 185–210 bp hypervariable segment.

The 15–25 bp region is only polymorphic when the comparison includes different loci and different supertypic groups (Fig. 3A). It is not polymorphic when alleles within a supertypic family are compared (Fig. 3B, C, and D). This indicates that the 15–25 bp region plays a role in distinguishing loci and supertypic groups rather than in restricted T-cell recognition.

Discussion

The similarity of DR gene restriction maps in different haplotypes within an HLA-DR supertypic group and the concomitant differences across supertypic groups indicate that within such a supertypic group the different haplotypes are evolutionarily related. We have compared the structure of the different *DR$_\beta$* chain genes within and across supertypic groups in order to determine the relationship of the various loci to each other. Our first observation is that the gene encoding the DRw53 specificity has characteristic structural features which correspond more closely to the *DR$_\beta$II* pseudogene in the *DRw52* haplotypes than to any of the active *DR$_\beta$* chain loci, including the *DR$_\beta$I* locus of the *DRw53* haplotypes. Comparison of first-domain se-

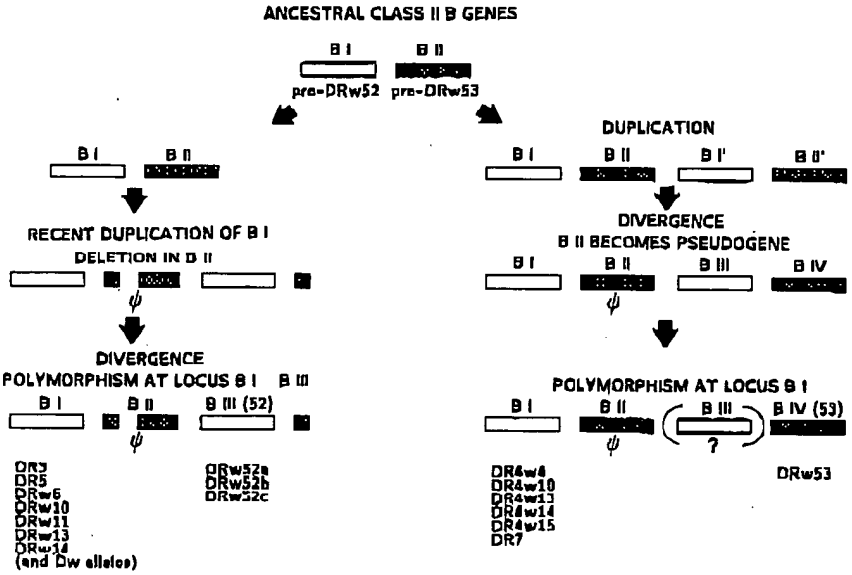

Fig. 4. Schematic representation of the evolutionary tree of the *DR$_\beta$* chain gene family. A two-locus family is postulated as the mammalian ancestral arrangement. The DRw52 lineage *(left)* has undergone a deletion of most of the ancestral *DR$_\beta$II* locus. The *DR$_\beta$I* locus and promoter region of the *DR$_\beta$II* locus were duplicated. These two events could have happened in opposite order or simultaneously. In the DRw53 lineage the ancestral *DR$_\beta$I-DR$_\beta$II* pair was duplicated. Whether locus *DR$_\beta$III* has been deleted or maintained will only be known when the linkage maps are available. Polymorphic variations were introduced at the *DR$_\beta$I* loci at a higher rate than at the other loci, thus establishing haplotypic (*DR$_\beta$I*) and supertypic (*DRw52* and *DRw53*) loci

quences of the active loci shows the corollary: the first locus of *DRw53* haplotype ($DR4_\beta I$) is more closely related to either of the two active *DRw52* loci ($DR_\beta I$ and $DR_\beta III$) than it is to the *DRw53* locus in its own haplotype.

These observations indicate that the *DRw53* and *DRw52* loci are not alleles in the classical sense. Based on these structural data, a limited evolutionary scheme is proposed here to explain the origin of *HLA-DR* polymorphism in these two supertypic groups (Fig. 4). We propose two ancestral DR_β chain genes, $DR_\beta I$ (pre-*DRw52*) in white in Figure 4, and $DR_\beta II$ (pre-*DRw53*) in black in Figure 4. In the DRw52 lineage (left), a deletion involved part of the $DR_\beta II$ locus and a duplication event took place involving the $DR_\beta I$ gene (in white in Fig. 4) and the remnant promoter region of the $DR_\beta II$ locus. This resulted in the two highly homologous $DR_\beta I$ and $DR_\beta III$ genes and in the truncated $DR_\beta II$ pseudogene found in the DRw52 lineage (Rollini et al. 1985). We postulate that the current DRw53 organization arose by an early gene duplication of the pair of ancestral DR_β chain loci resulting in four loci (Fig. 4, right). The ancestral $DR_\beta II$ locus became a pseudogene early, and has undergone extensive mutation (Larhammar et al. 1985). The fate of the third locus in the DRw53 lineage is not yet known as the complete linkage map has not been completed (Spies et al. 1985). Since four DR_β chain genes have been identified in certain *DRw53* haplotypes (Böhme et al. 1985), the *DRw53* locus (black in Fig. 4) should probably be referred to as $DR_\beta IV$. In both the DRw52 and w53 lineages, the different loci were submitted to variable mutation rates depending on their position on the chromosome, with the locus adjacent to the DR_α locus undergoing the least mutation. At this point, various alleles at locus $DR_\beta I$ (Fig. 4, bottom line) arose by different mechanisms, including gene conversion (Gorski and Mach 1986).

This evolutionary scheme accounts best for the structural data presented here. It implies that the *DRw53* locus and the $DR_\beta II$ locus of the DRw52 family share a common ancestor and thus can be considered alleles (black boxes). The $DR4_\beta I$ locus and the $DR_\beta I$ and $DR_\beta III$ loci of the DRw52 family share a common ancestor (white boxes). More importantly, the origin of the supertypic locus in each lineage, DRw52 and DRw53, is clearly different and thus the *DRw52* and *DRw53* loci cannot be considered alleles in the classical sense.

In the DRw53 lineage, the *DRw53* locus is identical in *DR4* and *DR7* haplotypes which differ in their $DR_\beta I$ locus and in their Dw specificities (Cairns et al. 1985, Gregersen et al. 1986a, b). In the DRw52 lineage, three alleles of the less polymorphic locus, *DRw52* or $DR_\beta III$, have recently been identified (Gorski and Mach 1986, and unpublished data). These alleles split the DRw52 group of haplotypes into the 52a, b, and c subgroups. Segregation studies using oligonucleotide probes specific for the *DRw52a* and *DRw52b* alleles further indicate that the haplotypic DR and Dw specificities are not encoded by the $DR_\beta III$ locus but rather by the $DR_\beta I$ locus (Gorski et al. 1987).

The DNA sequence comparisons have allowed us to map the positions of allelic differences. When true allelic comparisons are made, within a supertypic group, the polymorphism of the $DR_\beta I$ locus is characterized by a "hypervariable region" corresponding to bp 185–210 (amino acids 67–77). It is of interest that this segment has been implicated as the site of micro-recombination/gene conversion events, which directly affect the specificity and restriction of T-cell recognition (Gorski and Mach 1986). Interestingly, allelic comparisons of the $DR_\beta III$ locus in the DRw52 supertypic group show a very different pattern of polymorphic sites, with little or no differences in the "hypervariable region" at bp 185–210. This suggests that the mechanisms responsible for the allelic polymorphism at locus $DR_\beta I$ and locus $DR_\beta III$ are not the same. It also raises the question of the nature of T-cell recognition of the products of each of these loci and of whether these two DR products play similar or different roles in the immune response, in particular in restricted antigen presentation to T cells.

The differences in the structure of the two supertypic groups have major consequences for serological analysis. The *DRw53* locus does not have a corresponding allele in the DRw52 family. Therefore, heterozygosity within and between supertypic groups does not imply the same degree of structural differences. For example, a DR3, DRw6a individual is really DR3, DRw6a, DRw52a whereas a DR3,DR4 individual is DR3, DR4, DRw52a, DRw53. This difference in relative heterozygosity may prove to have important implications in the ability to respond to a wider selection of antigens and in the association of HLA class II with disease susceptibility. More importantly, it indicates that in HLA matching for organ transplantation, all HLA-DR mismatches are not of identical magnitude and consequence, especially when the supertypic loci (*DRw52* and *DRw53*) are taken into account. As shown in the example above, a single DR mismatch within a supertypic group can imply only a minor difference limited to the $DR_\beta I$ gene product, whereas any mismatch across supertypic groups involves the additional differences in the DR antigen encoded in the DRw52 and DRw53 supertypic loci. We have therefore proposed (Mach et al. 1986) that in addition to the *number* of mismatches, the *nature* of the mismatch might have important practical consequences in transplantation and should be taken into consideration.

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